

 THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE	UQ Animal Ethics Committee - Standard Operating Procedure LAB_050 Irradiation and Reconstitution in Mice (Expiry: March 2026) Institutional author: UQ Biological Resources AEC Reviewed & Approved: March 2025 SOP Expiry: March 2026	Version #2 Page 1 of 6
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LAB_050 Irradiation and Reconstitution in Mice (Expiry: March 2026)

I. OBJECTIVE

To describe the procedure for performing whole-body gamma irradiation for the purpose of complete, or partial, immuno-ablation in mice. The procedure also aims to describe the associated monitoring and care required to support animal wellbeing.

NB: The use of (*) indicates this statement is dependent on the facility procedures

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II. COMMENTS / RECOMMENDATIONS

- Irradiation presents a dose-dependent physiological challenge which is often significant; mice must be monitored at least daily for the first 14 days post-irradiation.
- Following total body irradiation (TBI) mice are immunocompromised and susceptible to infections previously considered clinically unimportant. Strict adherence to Specific Pathogen Free (SPF) procedures is required, and prophylactic antibiotics may be used at the investigator's discretion (antibiotic use details must be provided in the ethics application).
- Transplantation of haemopoietic stem cells ("reconstitution") must occur within 72 hours post-irradiation.
- If control animals are to be used (animals that are lethally irradiated but then do not undergo reconstitution), their use must be described and justified to the AEC, including clear considerations for replacement (e.g. use of data from historical controls) and reduction (e.g. use of as few as possible)
- Radiation doses may vary greatly, dependent on the model (see table 1 & 2, which gives some guidance on dosages). The specific dose, or dose range, must be described and justified to the AEC.
- "Lethal" irradiation (complete myeloablation) is a major physiological challenge; mice will display generalised symptoms of being "unwell" and can be expected to lose up to 25% of their body weight by day 7 post irradiation. Despite this impact, if reconstitution was successful symptoms should resolve and the mice will regain most of its weight during the following week. If symptoms are not obviously resolving by day 14 post irradiation the mouse should be euthanised, unless otherwise specifically advised by a facility veterinarian.
- Wherever appropriate the total irradiation doses should be fractioned (split across at least 2 sessions) to reduce the overall impact and associated morbidity to the mice (particularly gastrointestinal toxicity).
- When commencing a new model (or work with a novel strain), a pilot study should be conducted to determine the optimal radiation dose – assuming appropriate literature references are not available.

In relation to human safety:

- Facility and procedure appropriate PPE use is essential when handling laboratory rodents
- All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form. This procedure has particular risks of:
 - needle stick and mouse bite injury – take appropriate care
 - musculoskeletal injury when performed regularly – consider suitable ergonomic design wherever possible
- In the event of a spill follow facility emergency spill procedures relative to SDS details.

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III. EQUIPMENT

- PPE (*)
- Antibiotics – in drinking water <optional> (**)
- Irradiator (shielded gamma irradiator or x-ray irradiator) (*)
- Home cage +/- specific cage for use in the irradiator (*)
- Surface disinfectants (*)
- Needle (25-30G)
- Syringe
- Haemopoietic stem cells (or other transfusion product) (**)
- Sterile isotonic fluids (e.g. 0.9% saline)
- Weight scales

IV. PREPARATION

1. Monitor and record of the mouse's physical condition (including recording of pre-irradiation body weight). If the animal appears at all unwell it **should not** be irradiated.
2. If antibiotics are to be used (in drinking water) commence provision 3 days prior to irradiation. Throughout this period, record the volume of water consumed and compare it to prior volumes of water consumed, ensuring the mice are drinking sufficient volumes of the supplemented water. (see table 3, which provides options and comment on antibiotic treatment via drinking water)
3. Ensure all information provided to radiation technicians is correct, including project #, animal numbers and identifiers, irradiation dose and dose schedule. (**)

V. PROCEDURE

Irradiation:

1. If a specific cage is required for use in the irradiator (separate to the home cage), gently transfer the mice into this cage (as per SOP LAB_006 Handling and Restraint in Mice and Neonates).
2. Place the cage containing mice into the irradiator for the dose-dependent timeframe (usually <15 min). (**)
3. If the irradiation dose is being split, withdraw the mice from the irradiator for approximately 3 hours, prior recommencing with the second half of the irradiation dose. During this period of rest ensure the mice are held within their home cage in an appropriate holding area.
Fractionating the total radiation dose should be performed, wherever appropriate, to reduce the overall impact and associated morbidity to the mice (particularly gastrointestinal toxicity).
4. Following completion of irradiation, if a specific cage was used in the irradiator, return the mice to their home cage and disinfect any contaminated surfaces. (*)
5. Return the irradiated mice to their home rack and label their cage card to indicate date of irradiation and radiation dose received.

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Reconstitution:

6. Within 72 hours of irradiation, perform reconstitution by injection.
 - i. Adult mice: Usually this will occur within 12 to 48hrs of irradiation. Injection may occur as per LAB_030 Injections - Intravenous (IV) tail vein in Mice and Rats, or LAB_027 Injections - Retro-Orbital Injection in Mice.
 - ii. Neonatal mice: Dependent on the model, reconstitution may be appropriate immediately after irradiation. Injection may occur as per LAB_042 Injections - Intrahepatic in Neonates.
In some models neonates may not require irradiation prior to reconstitution (particularly when using immunocompromised strains).
7. If a high dose of irradiation has been used, consider treating mice with 0.5-1mL of sterile isotonic fluids subcutaneously throughout their acute period of recovery from irradiation (as per SOP LAB_017 Injections - Subcutaneous (SC) Injection in Mice and Rats). This may be performed initially at the same time as reconstitution (during the same animal handling session), then repeated every 24 hours as symptoms associated with radiation toxicity are observed or are expected to be observed.
8. Monitor mice at least daily until 14 days post irradiation.
Under some circumstances, monitoring through this period may be less frequent; e.g. established protocols that use low/moderate irradiation doses. If animals experience any morbidity or mortality, daily monitoring is required.

VI. REFERENCE INFORMATION

Table 1 | Overview of irradiation dose impacts, and their model implications.

The impacts of irradiation are relative to mouse strain (e.g. *scid* mice are relatively radiosensitive), age (neonates are more sensitive than aged mice, which are more sensitive than adult mice), and even sex (often males are more sensitive than females).

Irradiation dose (relative)	High dose	Low dose
Impacts to animal and its immune system	- Greater likelihood of achieving rapid, and complete myeloablation - Will cause greater irradiation-induced toxicity (including morbidity and mortality)	- Greater likelihood of incomplete ablation of the host's immune system: development of a mixed chimera - Irradiation-induced toxicity is still likely to be felt by the mouse (but of less severe impact)
Model implications	Required where the intention is to achieve rapid and complete chimerism (where the host's immune system becomes 100% donor-derived)	May result in delayed chimerism, failure of donor engraftment, or some other state of mixed chimerism - this may be the intention e.g. when developing models of graft vs host disease (GvHD) or performing transfer of leukaemia cells

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Table 2 | Overview of irradiation dosages, relative to mouse strain, and experimental protocol.

Mouse Strain	Irradiation Protocol	References
-	As a guide, complete myeloablation is likely to be achieved in any immunocompetent (adult) strain at, Single or fractionated dose: 700 – 1300 cGy	(1)
C57Bl/6 (similar level of radio-sensitivity to C3H and SJL)	Complete myeloablation (adults) Single dose: 950 – 1100 cGy Fractionated dose: 2 x 550 cGy Partial myeloablation (adults) Single dose: 350 – 600 cGy	(2-4)
BALB/c (more radiosensitive than C57Bl/6 mice)	Complete myeloablation (adults) Single dose: 600-800 cGy Fractionated dose: 2 x 400 cGy Partial myeloablation (adults) Single dose: <600 cGy	(5, 6)
scid (Highly radiosensitive)	Immunosuppression (adults) Single dose: 240 - 350 cGy (this dose is usually sufficient, however, some models will use up to 400 cGy) Neonatal pups (<3 days) for the purpose of developing a humanised mouse model Single dose: 100 cGy(7)	(7-10)

Table 2 references:

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Table 3 | Two broad spectrum antibiotic formulations commonly used for prophylaxis in irradiated mice.

The formulations listed assume provision via drinking water (ad libitum). In such circumstances, where a rodent's water supply is altered (e.g. medicated), fluid consumption must be monitored daily (i.e. by recording water bottle losses), for at least the initial period of habituation, until fluid consumption appears stable and comparable to premedicated fluid consumption. This is particularly important in irradiated mice because it is believed that illness can accentuate their already heightened sense of neophobia (i.e. avoidance of the novel water). This is one of the reasons antibiotics should commence prior to any symptoms developing in the mice (some research groups will commence antibiotics up to 3 days prior to irradiation).

Antibiotic use requires the following considerations:

- are mice consuming the medicated water, and are they consuming it in sufficient volumes to achieve an effective dose,
- the type of antibiotic(s) selected – it should be broad spectrum and empirically selected,
- in theory, “clean” mice that are strictly managed, following SPF procedures do not require antibiotics,
- some animals will have adverse reactions to antibiotics (particularly immunocompromised strains),

Use of antibiotics is at the researcher's discretion. For advice on antibiotic use, contact UQBR Veterinary Services

<uqbrvetservices@uq.edu.au>

Antibiotic	Logistics
Sulfamethoxazole and trimethoprim (TMS) Assuming commercial product contains 40mg sulfamethoxazole and 8mg trimethoprim / mL	Add 6mL of TMS to 250mL of standard drinking water. Cover the bottle (due to antibiotic light sensitivity). Re-suspend the solution daily by shaking the water bottle. Discard solution and refresh after 7 days.
Enrofloxacin Assuming commercial product contains 50mg enrofloxacin / mL	Add 2.5mL of enrofloxacin per 250mL of standard drinking water. Cover the bottle (due to antibiotic light sensitivity). Discard solution and refresh after 7 days.

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Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
#1	HS	18/02/2021	18/02/2024 superseded
#2	ABS	11/05/2022	11/05/2025

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